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Ozone Flux in 'Glycine max' (L.) Merr. Sites of Regulation and Relationship to Leaf Injury

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Hood and Dare cultivars of soybean, Glycine max (L.) Merr., vary in their foliar response to ozone. The physiological basis of this variation was investigated as a function of leaf age through an analysis of ozone flux data, leaf developmental morphology, and analogue modeling techniques. At all concentrations (0.25 - 0.58 ul l^{-1}) and exposure times (1-4 h), resistance to O_3 flux in the gas phase of the diffusive pathway (i.e., boundary layer and stomate) did not account fully for variation in pollutant uptake rates into the leaf interior. Ozone molecules experienced a residual resistance to diffusion that is not shared by effluxing water vapor molecules. Residual resistance to flux increased with pollutant concentration and exposure time and was associated with age-dependent differences in foliar O_3 response. Leaf morphology data, including stomatal frequency and the ratio of internal to external surface area, did not help explain cultivar or age-dependent differences in O_3 flux. The extent of foliar injury was not consistently related to the magnitude of O_3 flux into the leaf interior. An analysis of the residual resistance to O_3 flux suggests that the gas and liquid phase pathways for O_3, water vapor, and carbon dioxide are not identical.

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Ozone Flux in *Glycine max* (L.) Merr.: Sites of Regulation and Relationship to Leaf Injury

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Summary. Hood and Dare cultivars of soybean, *Glycine max* (L.) Merr., vary in their foliar response to ozone. The physiological basis of this variation was investigated as a function of leaf age through an analysis of ozone flux data, leaf developmental morphology, and analogue modelling techniques. At all concentrations (0.25–0.58 $\mu l\ l^{-1}$) and exposure times (1–4 h), resistance to O_3 flux in the gas phase of the diffusive pathway (i.e., boundary layer and stomate) did not account fully for variation in pollutant uptake rates into the leaf interior. Ozone molecules experienced a residual resistance to diffusion that is not shared by effluxing water vapor molecules. Residual resistance to O_3 flux increased with pollutant concentration and exposure time and was associated with age-dependent differences in foliar O_3 response. Leaf morphology data, including stomatal frequency and the ratio of internal to external surface area, did not help explain cultivar or age-dependent differences in O_3 flux. The extent of foliar injury was not consistently related to the magnitude of O_3 flux into the leaf interior. An analysis of the residual resistance to O_3 flux suggests that the gas and liquid phase pathways for O_3, water vapor, and carbon dioxide are not identical.

Introduction

Gaseous exchange is a central issue in understanding whole-plant response to air pollution stress. Pollutant flux is governed by the ratio of the gas's concentration gradient to the pathway's diffusive resistance. Any physical or chemical factor that enhances or impedes flux will, in turn, influence the cellular concentration of the pollutant or its derivative. Runeckles (1974) proposed the term "effective pollutant dose" to discriminate between the dose in ambient air and leaf interior, since the latter more accurately depicts the level of stress to which the plant responds. Given the substantial variation in pollutant flux as the environment or genotype differs, dissimilar plant responses may be a consequence of unequal uptake rates of the pollutant. Flux differences are commonly inferred from measures of either the ambient pollutant concentration or diffusive resistance to water vapor efflux. However, this approach does not account for other factors that also influence the pathway's diffusive resistance or the pollutant's chemical potential gradient (Tingey and Taylor 1981).

Regulatory sites for diffusion of water vapor and carbon dioxide occur in both the gas and liquid phase (Nobel 1974) and involve physical resistances, per se, or source-to-sink components of the chemical potential gradient. For H_2O, only gas phase components (i.e., stomatal and boundary layer resistances, H_2O concentration differential) control the rate of transpiration. In contrast, influxing CO_2 molecules experience both gas and liquid phase restrictions (Gaastra 1959) that are a consequence of the longer, metabolically more complex CO_2 diffusion pathway. Depending upon the species and environmental conditions, the predominant site controlling CO_2 assimilation may be in either phase (Gaastra 1959; Cowan and Troughton 1971).

Plant genotype and environmental growth conditions influence plant response to air pollution stress, and pollutant flux differences may be the source of this variation. Pollutant flux may be regulated at the boundary layer, stomate, and sites within the leaf interior (Mansfield 1973; Unsworth et al. 1976). In the case of the latter, Turrell (1942) hypothesized that larger intercellular space and internal surface area enhance pollutant flux, while suberization of mesophyll cell walls and greater tissue compaction reduce flux (Bobrov 1955; Glater 1956). Barton et al. (1980) speculated that the mass flow of effluxing H_2O molecules in the transpiration stream may impede the diffusion of influxing pollutant molecules on mesophyll cell surfaces. Thus, sites for regulation of pollutant uptake may reside in both gas and liquid phases.

Hood and Dare cultivars of *Glycine max* vary in their foliar response to ozone (Tingey et al. 1972), and the greater resistance in Hood could not be attributed to reduced pollutant flux (Yingjajaval 1976). In both cultivars, susceptibility varies with leaf age, increasing to a maximum during the latter stages of rapid leaf expansion (Tingey et al. 1973). Using these consistent leaf age and cultivar differences in O_3 foliar response, we investigated the relationships between leaf injury, pollutant flux, and leaf morphology.

Materials and Methods

Leaf Morphology. *Glycine max* (L.) Merr. cultivars (Hood and Dare) were grown in a jiffy mix:perlite (1:2; v/v) potting mix and watered daily with North Carolina State University Phytotron Nutrient Solution (Downs and Hellmers 1975). Both cultivars were grown within the same environmental chamber with the following conditions: 12 h light period of PAR quantum flux of 430 $\mu E\ m^{-2}\ s^{-1}$ (the middle of the dark period was interrupted with incandescent light at 100 $\mu E\ m^{-2}\ s^{-1}$ for 3 h); day/night temperature regime of 28/21° C; and relative humidity of $70 \pm 10\%$. When plants (3 from each cultivar) exhibited plastochron indices (Lamoreau et al. 1978) of approximately 6–7,

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leaf areas were determined for each trifoliate. Two leaf discs (6.5 mm diam.) were taken from the center leaflet of each leaf at nodes 2 through 5. The discs (2) were sampled from the region equidistant from the midvein and leaf margin and intermediate between the base and tip. Each trifoliate was assigned a leaf plastochron index (LPI) value using a reference length of 20 mm. Discs were fixed immediately in 3% glutaraldehyde in 0.025 M phosphate buffer at pH 6.8 and 10% DMSO for 12–24 h at 0° C, dehydrated, and embedded in glycol methacrylate (Feder and O'Brien 1968). Transverse and paradermal sections (2–3 μm thick) were cut on a JB-4 Sorvall microtome, mounted, and stained with toluidine blue O. Photomicrographs were taken with a Leitz Orthomat II using Kadachrome II and monochrome negative films. Both photomicrographic images were enlarged to permit tracings and measurement of internal morphological features. Abaxial and adaxial stomatal frequency was assessed from leaf surface impressions (Williams 1973) taken on four additional plants of known LPI values in the range 2–5. Adaxial and abaxial leaf porosity was determined using a pressure porometer (Tingey et al. 1976).

Cell height, cell volume, internal and external leaf areas, ratio of internal to external surface area, intercellular space volume, and surface area to volume ratios were calculated using Turrell's methods (1936, 1942). The ratio of internal to external surface area was doubled for consistency with the Ames/A ratio (Nobel et al. 1975). The paraveinal cell layer, a unique feature of *G. max* (Fisher 1967; Weston and Cass 1973) was treated as a distinct tissue as were the spongy mesophyll, upper and lower palisades, and lower epidermis. For each leaf disc, 20 different sample images were studied.

For each measure of leaf morphology, the data were transformed to their respective logarithms to normalize the variance (Snedecor and Cochran 1967) since growth data are commonly distributed lognormally. Analysis of variance was performed so that variation accountable to LPI and cultivar could be partitioned. Unless otherwise indicated, data are presented as geometric means (Snedecor and Cochran 1967).

Gaseous Exchange. Plants were cultured as described in the leaf morphology section. Gaseous exchange measurements were taken when the first trifoliate leaves had LPI's of 3.0 ± 0.2 and 5.5 ± 0.4. The two LPI intervals (ca. 3 and 5.5) represented leaves of $70 \pm 10\%$ and 95% expansion, respectively (Yingjajaval 1976).

A single leaf, gas-exchange chamber (Bierhuzien and Slayter 1964) was used to measure gaseous fluxes when leaves were exposed to O_3 concentrations of 0.0, 0.25, 0.34, 0.40, and 0.58 μl l^{-1} for 4 h. During the exposure the following environmental factors were held constant: PAR quantum flux of 680 μE m^{-2} s^{-1}, outlet air temperature of 28° C, outlet relative humidity of 65%, and outlet CO_2 concentration of 330 ± 30 μl l^{-1}. Ozone was measured using a chemiluminescent analyzer, while CO_2 and H_2O vapor were measured with an infrared analyzer and differential psychrometer, respectively (Yingjajaval 1976). Gaseous fluxes were calculated from measures of the flow rate, differential gas concentrations between inlet and outlet ports (taking into account loss to chamber walls) and leaf area. Boundary layer resistance to H_2O vapor efflux was calculated from the energy budget approach (Sestak et al. 1971). In addition to a residual resistance to O_3 uptake, we calculated O_3 flux to the leaf surface, leaf interior, and mesophyll cell surface using Yingjajaval (1976) data on total leaf flux of O_3 and gas-phase resistance (boundary layer and stomate) to H_2O vapor loss. These data were then related to leaf morphology.

The analysis of O_3 flux as a function of time and pollutant concentration for each age class and cultivar was accomplished

Table 1. Ozone flux, concentrations and resistances derived from analogy of pollutant flux to Ohm's Law

Component	Symbol	Units	Solution	Comments
1. Total O_3 flux to the leaf	J_{TOTAL}	ng m^{-2} s^{-1}	Experimentally measured	
2. O_3 flux to leaf surface	$J_{SURFACE}$	ng m^{-2} s^{-1}	Experimentally measured	
3. O_3 flux to leaf interior	$J_{INTERNAL}$	ng m^{-2} s^{-1}	$J_{INTERNAL} = J_{TOTAL} - J_{SURFACE}$	
4. Ambient O_3 concentration	$C_a^{O_3}$	ng m^{-3}	Experimentally measured	
5. Boundary layer resistance to water vapor efflux	$R_a^{H_2O}$	s m^{-1}	Experimentally measured	
6. Boundary layer resistance to O_3 flux	$R_a^{O_3}$	s m^{-1}	$R_a^{O_3} = R_a^{H_2O} * 1.63$	Assumes resistance is a function of the ratio of the diffusion coefficients of the two gases in air
7. Stomatal resistance to water vapor efflux	$R_s^{H_2O}$	s m^{-1}	Experimentally measured	
8. Stomatal resistance to O_3 flux	$R_s^{O_3}$	s m^{-1}	$R_s^{O_3} = R_s^{H_2O} * 1.63$	Same assumption as $R_a^{O_3}$
9. Leaf surface resistance to adsorption of O_3	$R_e^{O_3}$	s m^{-1}	$J_{SURFACE} = (C_a^{O_3} - C_e^{O_3})/(R_a^{O_3} + R_e^{O_3})$ $R_e^{O_3} = (C_a^{O_3}/J_{SURFACE}) - R_a^{O_3}$	Assumes O_3 is destroyed on leaf surface after adsorption. C_e is the concentration on the epidermis; $C_e = O$
10. Ozone concentration at the leaf surface	$C_c^{O_3}$	ng m^{-3}	$J_{TOTAL} = (C_a^{O_3} - C_c^{O_3})/R_a^{O_3}$ $C_c^{O_3} = C_a^{O_3} - (J_{TOTAL} * R_a^{O_3})$	
11. Stomatal resistance to O_3 flux (model)	$R_s^{O_3}$	s m^{-1}	$J_{INTERNAL} = (C_c^{O_3} - C_i^{O_3})/R_s^{O_3}$ $J_{INTERNAL} = C_c^{O_3}/R_s^{O_3}$ $R_s^{O_3} = C_c^{O_3}/J_{INTERNAL}$	Assumes as above that O_3 is destroyed within the leaf; $C_i = O$ $R_s^{O_3}$ is derived from the model
12. Residual resistance to O_3 flux	$R_r^{O_3}$	s m^{-1}	$R_r^{O_3} = R_s^{O_3} - R_s^{O_3}$	Compare $R_s^{O_3}$ derived in (/–/ 11) with measured $R_s^{O_3}$ (/–/ 8); if (/–/ 11) is greater than (/–/ 8), O_3 experiences a positive residual resistance

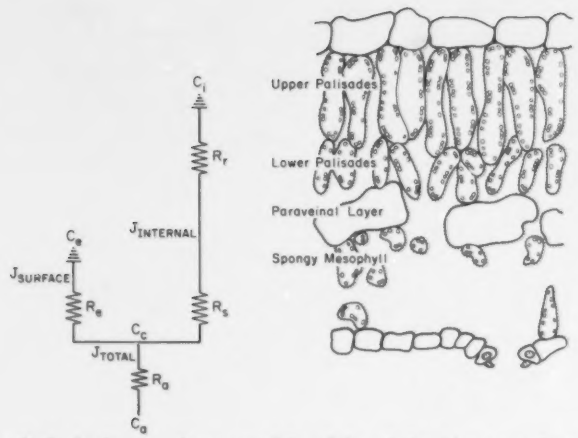

Fig. 1. Analogue resistance model and its relationships to soybean leaf morphology. Abbreviations: C_a = ambient O_3 concentration; C_c = O_3 concentration at leaf surface; C_e = O_3 concentration on leaf surface; C_{ic} = O_3 concentration in the intercellular space of leaf interior; C_i = O_3 concentration in solution in, or on, cells of leaf interior; R_a = boundary layer resistance; R_e = resistance to adsorption onto leaf surface; R_s = stomatal resistance; R_r = residual resistance. J_{TOTAL} is combined O_3 flux to the leaf surface ($J_{SURFACE}$) and interior ($J_{INTERNAL}$). Units: concentration – ng m^{-3}; resistance – s m^{-1}; flux – ng m^{-2} s^{-1}

using analogue techniques (Gaastra 1959) as has been done for several air pollutants (Mansfield 1973; Bennett et al. 1973; Unsworth et al. 1976; O'Dell et al. 1977; Black and Unsworth 1980). As with CO_2 assimilation (Gaastra 1959), the technique can assess the influence of boundary layer and stomatal resistances on O_3 flux, and any residual variation is attributed to physiological and biochemical processes affecting either the chemical potential gradient or pathway's diffusive resistance. This residual is analogous to Gaastra's mesophyll resistance to CO_2 assimilation ($R_m^{CO_2}$). Hereafter, these unexplained sources of variation in O_3 flux not attributed to the sum of boundary layer and stomatal resistance are collectively called a residual resistance and have units of s m^{-1}. The series of equations used and their derivation (Table 1) is based upon a resistance analogue model (Fig. 1).

Results

Leaf Morphology

External Morphology. External leaf area expands from approximately 25 to 100% as the LPI increases from 2–5 (Table 2). Leaf expansion rates are similar for both cultivars, with maximum expansion rates occurring between LPI 2–3, when the leaf attains approximately 70% of its final area. In both cultivars the abaxial surface has twice as many stomates as the adaxial (Table 2). For both leaf surfaces and at equivalent LPI's, Hood has a significantly higher stomatal frequency than Dare. However, relative stomatal resistance is essentially unchanged as leaves expand and is comparable between cultivars (Table 2). Hood exhibits 2–3 times less foliar injury than Dare at the same LPI, while maximum leaf injury occurs at LPI 3 in both cultivars.

Internal Morphology. Leaf mesophyll thickness increases with age, with Dare leaves tending to be thicker than Hood (Table 2). The differences in average thickness are 7 and 32 μm at LPI 2 and 5, respectively. However, the percentage that each tissue type contributes to leaf thickness is similar for the two cultivars and is constant between LPI 2–5: upper palisades, 35%; lower

Table 2. Morphological and physiological leaf features of Hood and Dare cultivars of *Glycine max*[a]

Leaf Feature	Leaf age (LPI)								LSD[d]	SGD[e]
	Hood				Dare					
	2	3	4	5	2	3	4	5		
Leaf expansion[c] (%)	25	70	87	100	28	68	88	100	8.3	–
Foliar injury[c] (%)	2	23	21	19	21	65	47	44	14	–
Stomatal frequency (stomata mm^{-2})										
Adaxial	100	77	64	52	81	60	58	48	–	1.19
Abaxial	189	157	133	118	174	134	119	101	–	1.11
Relative stomatal resistance[c]	4.7	4.4	4.3	4.3	4.5	4.2	4.3	4.4	0.4	–
Leaf mesophyll thickness (μm)	117	145	154	150	124	158	178	177	–	1.16
Total intercellular airspace[b] ($\mu m^3 \times 10^3$)	350	537	577	553	329	449	593	661	–	1.23
Total cellular volume[b] ($\mu m^3 \times 10^3$)	442	511	490	510	505	682	657	596	–	1.13
Total exposed internal cell surface area[b] ($\mu m^2 \times 10^3$)	130	133	124	129	133	150	157	131	–	1.04
Ames/A	18.0	18.3	17.0	17.7	18.7	21.2	21.8	18.6	–	1.10

[a] Each LPI contains data for a range of ±0.5 LPI units of the given unit. Data for leaf expansion, foliar injury and relative stomatal resistance are arithmetic means; all other leaf feature data are geometric means

[b] The volume and area represent the volume and area of the leaf in one microscopic view of approximately 7,100 μm^2

[c] Data are arithmetic means from Tingey et al. (1976)

[d] Least Significant Difference ($p = 0.05$)

[e] Standard Geometric Deviation or the value multiplied by or divided into geometric mean ($n = 3$) to obtain 95% estimation limits (Snedecor and Cochran 1967)

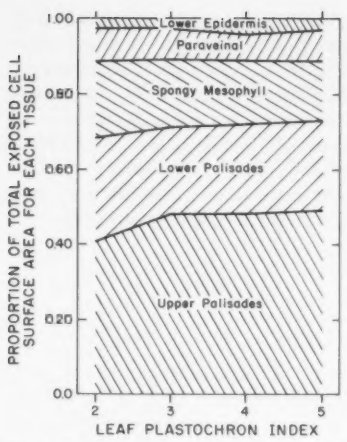

Fig. 2. Proportion of total exposed cell surface area contributed by each tissue layer as a function of LPI. Data are presented as means of the two cultivars because differences between cultivars are not statistically significant

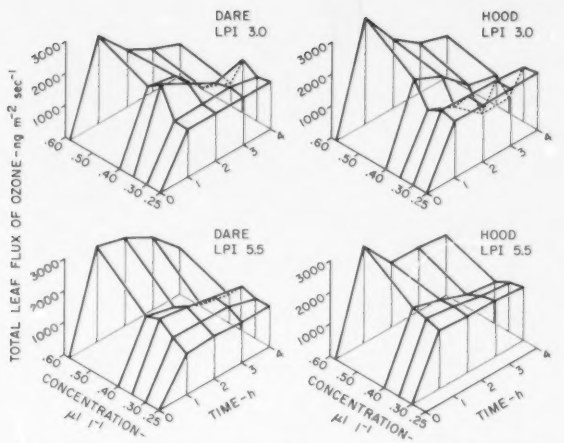

Fig. 3. Total leaf flux (J_{TOTAL}) of O_3 in Hood and Dare cultivars at LPI 3.0 and 5.5 as a function of O_3 concentration and exposure time. Flux data at 0.25 µl l^{-1} O_3 for Hood LPI 5.5 were not recorded

palisades, 25%; paraveinal, 12%; and spongy mesophyll, 28%. Total intercellular air space increases with leaf age, and although intercellular space increases to a larger extent in Dare, the differences are not statistically significant (Table 2). Total cellular volume of Dare is larger than Hood, but the proportion of cellular volume for each cell type is similar between cultivars indicating that all cell types are larger in Dare than Hood. Total internally exposed cell surface area is greater in Dare with the greatest differences (13–27%) occurring at LPI's 3 and 4 (Table 2). The proportion of total exposed cell surface contributed by each cell type is similar for each cultivar and exhibits only slight changes with LPI (Fig. 2). The upper palisade contributes between 41 and 48% of the total with the lower palisade and spongy mesophyll contributing approximately 25 and 28%, respectively. The larger cell surface area of Dare is reflected in the significantly larger Ames/A ratio (Table 2), indicating that Dare has more internal cell surface area exposed to intercellular space.

Ozone Flux

Total Leaf Flux of O_3 (J_{TOTAL}): J_{TOTAL}, the sum of leaf adsorption and absorption, of O_3 varies with pollutant concentration and duration of exposure (Fig. 3). Maxima of approximately 3,000 ng m^{-2} s^{-1} are recorded at 0.58 µl l^{-1} O_3 within the first hour, after which J_{TOTAL} declines in each cultivar and at both leaf ages. In contrast, J_{TOTAL} at the lower concentrations remains essentially uniform throughout the 4 h exposure. At the first hour of exposure, J_{TOTAL} increases with concentration. At longer time intervals (2–4 h), flux decreases consistently with time at 0.58 µl l^{-1}. After 0.58 µl l^{-1} for 4 h, J_{TOTAL} is equal to or less than the flux at 0.25 µl l^{-1} for all cultivars and leaf ages. Thus there is a lack of proportionality between J_{TOTAL} and the ambient O_3 concentration over time.

Leaf Surface Flux of O_3 ($J_{SURFACE}$): $J_{SURFACE}$ increases linearly with ambient concentration at each time interval except at 3 and 4 h in the younger leaves (Fig. 4). Irrespective of O_3 concentration, $J_{SURFACE}$ is greatest at the first hour and declines thereafter. For younger leaves, surface adsorption of O_3 is not detected after 3 h at any concentration. At all exposure times and

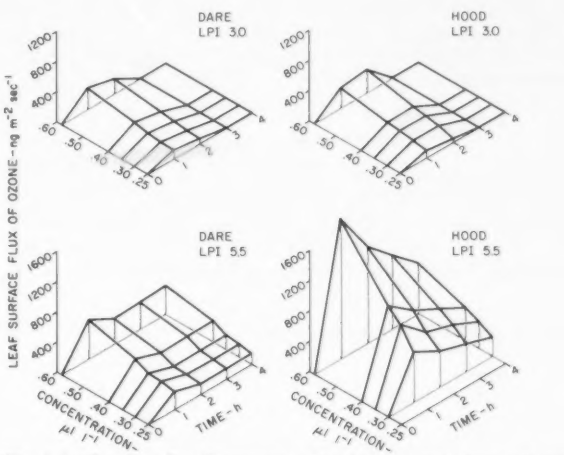

Fig. 4. Leaf surface flux ($J_{SURFACE}$) of O_3 in Hood and Dare cultivars at LPI 3.0 and 5.5. Flux is shown as a function of O_3 concentration and exposure time

concentrations $J_{SURFACE}$ is similar at LPI 3.0 for Dare and Hood. In contrast, $J_{SURFACE}$ at LPI 5.5 is much greater for Hood than Dare.

Internal Leaf Flux of O_3 ($J_{INTERNAL}$). $J_{INTERNAL}$ is the major fraction of J_{TOTAL}, and it is never less than 70% of J_{TOTAL} except in Hood at LPI 5.5 (Fig. 5). Irrespective of the ambient O_3 concentration, at the end of the 4 h exposure $J_{INTERNAL}$ is strikingly similar. At the highest concentration (0.58 µl l^{-1}), $J_{INTERNAL}$ decreases with time. This contrasts with the lower concentrations where $J_{INTERNAL}$ is unchanged over the 4 h exposure.

To recognize patterns in $J_{INTERNAL}$ associated with either age or cultivar effects, the response surface is divided into O_3 flux at low (0.25–0.40 µl l^{-1} O_3) and high (0.58 µl l^{-1}) concentrations. A comparison of cultivar effects shows that at equivalent ages $J_{INTERNAL}$ for Hood is either greater than (LPI 3.0, high and low conc.; LPI 5.5, high conc.) or equal to (LPI 5.5, low conc.) O_3 flux in Dare. Younger leaves absorb more O_3 per leaf area than the older ones at the lower concentrations.

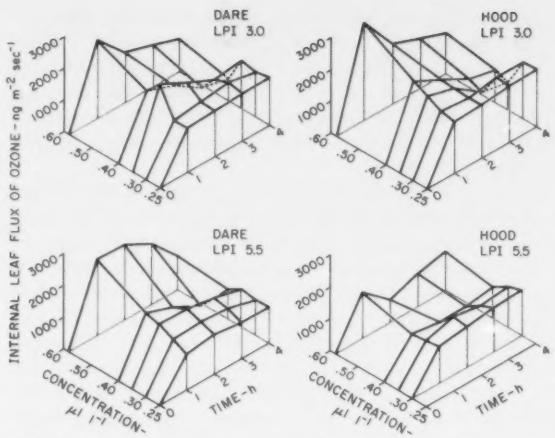

Fig. 5. Internal leaf flux ($J_{INTERNAL}$) of O_3 in Hood and Dare cultivars at LPI 3.0 and 5.5. Flux is shown as a function of O_3 concentration and exposure time

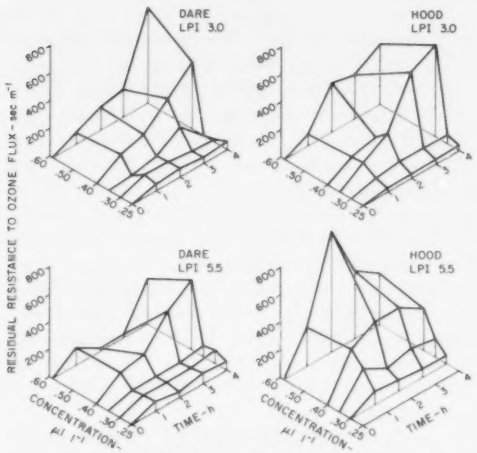

Fig. 6. Residual resistance ($R_r^{O_3}$) in Hood and Dare cultivars at LPI 3.0 and 5.5. $R_r^{O_3}$ is shown as a function of O_3 concentration and exposure time. Data for $R_r^{O_3}$ for Hood LPI 3.0 from 3–4 h at 0.40 µl l^{-1} are missing. Therefore $R_r^{O_3}$ values are not calculated

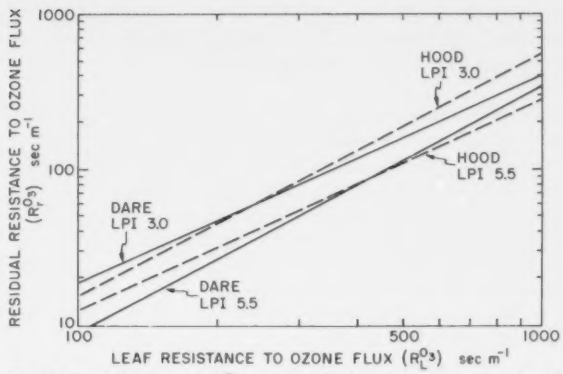

Fig. 7. Contribution of $R_r^{O_3}$ to total leaf resistance to O_3 flux for each cultivar at LPI 3.0 and 5.5. Each line is derived from linear correlation analysis

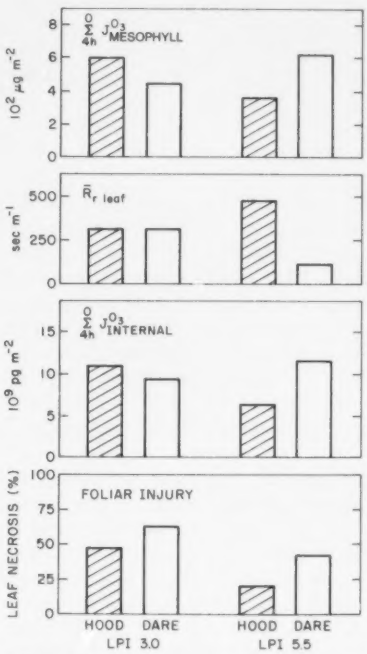

Fig. 8. Relationship among foliar injury, $J_{INTERNAL}$, $J_{MESOPHYLL}$, and $R_r^{O_3}$ for Hood and Dare after 4 h exposure to 0.58 µl l^{-1} O_3. Flux values are cumulative over the 4 h exposure, while $R_r^{O_3}$ is the arithmetic mean of the four hourly values

Since Ames/A relates internal to external surface area, O_3 flux per unit of exposed cellular surface area of the leaf interior is calculated as $J_{MESOPHYLL} = J_{INTERNAL} \cdot (Ames/A)^{-1}$. $J_{MESOPHYLL}$ varies 4-fold from 30–130 ng m^{-2} s^{-1}. The lowest cell surface flux values occur consistently after 4 h of exposure at intermediate O_3 concentrations (0.3–0.4 µl l^{-1}) while the highest values are at 0.58 µl l^{-1} after 1 h. Since Ames/A is constant the patterns of response for $J_{MESOPHYLL}$ as a function of O_3 concentration and time do not differ from $J_{INTERNAL}$ for each cultivar and LPI. Moreover, the minor variation in Ames/A between cultivars (5–15%) and LPI classes (3–10%) dictates that $J_{MESOPHYLL}$ follows closely $J_{INTERNAL}$. The generally higher $J_{INTERNAL}$ values for Hood versus Dare coupled with the respectively lower Ames/A ratios in Hood (5–15%) means that O_3 flux per unit of cell surface area is lower for Dare (O_3 sensitive) than Hood (O_3 resistant). At the lower O_3 concentrations ($\leqq$0.40 µl l^{-1}), these same factors tend to reduce the differences in O_3 flux per unit area of cell surface since younger leaves absorb more O_3 than older ones, yet have higher (3–10%) Ames/A ratios.

Residual Resistance to O_3 Flux ($R_r^{O_3}$). Residual resistance occurs at all O_3 concentrations and time intervals (Fig. 6). At low O_3 concentrations ($<$0.3 µl l^{-1}), $R_r^{O_3}$ is less than 100 s m^{-1} and remains unchanged after 4 h of exposure. At higher concentrations ($\geqq$0.4 µl l^{-1}), $R_r^{O_3}$ increases with time and concentration, exhibiting maxima from 530 to 930 s m^{-1}. As one of several determinants of O_3 uptake into the leaf interior, $R_r^{O_3}$ accounts for 10–50% of the leaf's total resistance ($R_L^{O_3}$) to O_3 flux (Fig. 7). Minimum contribution (10–20%) of $R_r^{O_3}$ to the leaf's diffusive resistance occurs when $R_L^{O_3}$ ranges from 100–400 s m^{-1}, while

the percentage increases to 25–50% at $R_L^{O_3}$ values approaching 1,000 s m^{-1}. $R_r^{O_3}$ tends to be a larger component of $R_L^{O_3}$ in the younger leaves of both cultivars (Fig. 7).

Relationship Among Injury, Flux, and $R_r^{O_3}$. Since acute foliar injury differences in Hood and Dare are more pronounced at O_3 concentrations exceeding 0.50 µl l^{-1}, the relationship among injury, $J_{INTERNAL}$, $J_{MESOPHYLL}$, and $R_r^{O_3}$ at 0.58 µl l^{-1} is presented graphically (Fig. 8). At LPI 3.0 the more resistant Hood absorbs 19% more O_3 into the leaf interior but exhibits 65% less injury than Dare. On a per cell surface area basis, O_3 flux is 26% more in Hood than Dare; however, $R_r^{O_3}$ differs by only 11%. At LPI 5.5, foliar injury continues to differ by 60% between the cultivars, although the relationship among injury, O_3 flux and $R_r^{O_3}$, is reversed: $J_{INTERNAL}$ and $J_{MESOPHYLL}$ are 44 and 41% higher respectively for Dare than Hood, and $R_r^{O_3}$ averages 300% higher in Hood than Dare.

The absence of consistent relationships among injury, O_3 flux and $R_r^{O_3}$, is further reflected in age-associated response to O_3 (Fig. 8). In Hood the more resistant older leaves absorb less O_3 ($J_{INTERNAL}$, 38%; $J_{MESOPHYLL}$, 40%) and have a 50% higher $R_r^{O_3}$. Conversely, in Dare $J_{INTERNAL}$ and $J_{MESOPHYLL}$ are 24 and 40% higher respectively in the more resistant older leaves, while $R_r^{O_3}$ is 60% less.

Discussion

If $J_{INTERNAL}$ is the most important factor underlying plant response to O_3, maximum pollutant flux should correspond to the most susceptible leaf ages and cultivars. In Hood, the more resistant older leaves absorb 38% less O_3 per unit leaf area at 0.58 µl l^{-1} than the younger more sensitive leaves. In contrast, the older foliage in Dare absorbs 24% more O_3 than the younger leaves. An analogous inconsistency occurs between cultivars. At LPI 5.5, $J_{INTERNAL}$ is more in the resistant Hood, while at LPI 3.0 the more sensitive Dare absorbs more O_3. Thus, the extent of foliar injury in *G. max* is not necessarily correlated with $J_{INTERNAL}$.

Variation in O_3 uptake into the leaf interior is commonly implicated as the mechanism underlying plant-to-plant differences in response to O_3 (Engle and Gabelman 1966; Butler and Tibbitts 1979). However, most techniques do not measure O_3 flux per se, but rather assume that the resistances of the countercurrent pathways for H_2O vapor and O_3 are comparable. McLaughlin and Taylor (1981) found that $J_{INTERNAL}$ is not necessarily proportional to the ambient O_3 concentration. Townsend (1974) and Evans and Ting (1974) measured total leaf flux of O_3 and found no consistent correlation between J_{TOTAL} and the extent of foliar injury. Our results, which provide a measure solely of $J_{INTERNAL}$, show no relationship between flux and injury in *G. max*. Thus, biochemical processes within the mesophyll cell are responsible in part for leaf age and cultivar differences in O_3 response in soybean. This may involve either liquid phase scavenging events or homeostatic processes that influence repair of, or compensation for, perturbed cellular structure or function (Tingey and Taylor 1981). In the terminology of Levitt (Levitt 1980; Taylor 1978), these differences result from either strain avoidance or strain tolerance.

In order for O_3 to experience leaf resistance qualitatively comparable to effluxing H_2O molecules, the pathways of the two gases must be equal. Since most of the H_2O molecules vaporize from peristomal sites (Tanton and Crowdy 1972; Tyree and Yianoulis 1980), this would necessitate that 70–90% of the O_3 molecules be deposited on surfaces of the substomatal cavity. For pollutants that are not readily soluble in water (O_3 is roughly one-third as soluble in H_2O as CO_2), this seems unlikely. Thus, the mean length of the diffusion path for the population of O_3 molecules is longer than that for H_2O vapor. As a consequence, leaf resistance data specific for H_2O may not provide sufficient information from which to estimate accurately total leaf resistance to O_3.

The tendency for most of the O_3 molecules to diffuse into the mesophyll tissue of the leaf interior is similar to the gas-phase pathway for CO_2. Only 10–20% of the influxing CO_2 molecules are deposited within the substomal chamber while the remainder diffuse further into the leaf interior and are extracted onto cell surfaces of the spongy and palisades mesophyll tissue (Cowan 1977; Rand 1977). Along the CO_2 diffusion path, deposition to cell surfaces can reduce progressively the CO_2 concentration within the intercellular space; however, the concentration drop between the stomatal pore and deep within the mesophyll tissue is estimated to be only 10–15% (Yocum and Lommen 1975; Rand 1978). Since O_3 is even less soluble in water than CO_2, the gas-phase pathway for O_3 should be more similar to that of CO_2 than H_2O.

The analagous gas-phase pathway for CO_2 and O_3 was extended by Yingjajaval (1976) to include events in the liquid phase. She assumed that, from the source in ambient air to sites of carboxylation, the two gases follow comparable pathways. However, the dissimilar biochemistry of the two gases makes this assumption unlikely (Tingey and Taylor 1981). Yingjajaval (1976) attributed decreasing CO_2 assimilation rates at high O_3 concentrations to an increasing CO_2 compensation point that affected the concentration gradient. Similarly, she proposed that differences in $J_{INTERNAL}$ for O_3 between Hood and Dare were a consequence of unequal numbers of O_3 deposition sites on mesophyll cell surfaces. The ratio of internal to external surface area (Ames/A) can be an important determinant of CO_2 flux (Nobel et al. 1975). However, our morphological data show that the more resistant Hood cultivar had greater O_3 uptake rates but consistently lower Ames/A ratios than the Dare cultivar at comparable LPI's. Thus factors other than the number of sites for O_3 deposition within the leaf interior are regulating pollutant flux.

Irrespective of O_3 concentration, duration of exposure, or cultivar, O_3 molecules experienced a residual resistance component not shared by effluxing H_2O molecules. This residual was more pronounced with higher O_3 concentrations and exposure times and accounted for 10–50% of the leaf's total diffusive resistance. Others have proposed the existence of a resistance to O_3 flux in addition to R_a+R_s. Wesely et al. (1978) and O'Dell et al. (1977) referred to it as a mesophyll resistance, while Leuning et al. (1979) offered the term chemical resistance. Implicit in Hill's (1971) conclusion that differences in pollutant flux among gases are due, in part, to the pollutants' solubility in water is a recognition that internal factors may also influence pollutant uptake. We report $R_r^{O_3}$ ranging from 70 to 900 s m^{-1}. Wesely et al. (1978) estimated $R_r^{O_3}$ values from 300 to 400 s m^{-1} from field estimates of flux to *Zea mays* canopy, while Leuning et al. (1979) assumed $R_r^{O_3}$ to be indistinguishable from zero. Using Hill's (1971) data on pollutant flux to alfalfa, Heath (1980) concluded that $R_r^{O_3}$ was 60 s m^{-1}.

Since Ames/A relates internal to external leaf area, O_3 flux per unit cell area of the leaf interior ($J_{MESOPHYLL}$) can be calculated as the ratio of $J_{INTERNAL}$ to Ames/A. Assuming that cells of the leaf interior are similar to palisade cells (diam. 7 µm and height 30 µm) and cylindrical, each cell possesses a maximum surface area of 736 μm^2. After 4 h of exposure to 0.25 µl l^{-1}

O_3, 1.51×10^{-14} moles (9.09×10^9 molecules) of O_3 would be deposited per cell in Dare at LPI 3.0. The cellular flux of O_3 in Hood LPI 3.0 at 0.58 $\mu l\, l^{-1}$ O_3 for 4 h is 2.32×10^{-14} moles or 1.40×10^{10} molecules. These calculations are upper-level estimates of O_3 flux per cell since not all cell surfaces front intercellular space. These values also represent maximum O_3 concentrations per cell; realistic concentrations are likely to be less due to O_3 scavenging reactions. Based upon data from Craker and Starbuck (1973), Heath (1975) calculated that lethal O_3 levels per cell in tobacco leaves are 4×10^{-14} moles.

We have investigated some of the factors that influence O_3 flux into the leaf interior and under the hypothesis that pollutant flux as influenced by leaf morphology is directly linked to the extend of injury. In comparison to the ambient pollutant concentration, $J_{INTERNAL}$ is a more direct measure of the pollutant dose to which a plant responds (Runeckles 1974). However, the extent of injury does not necessarily follow from $J_{INTERNAL}$. This is evidence that physiological and biochemical processes other than pollutant uptake influence O_3 response.

The movement of O_3 from ambient air to intracellular perturbation sites involves events both in the gas and liquid phases. Variation in gas-phase resistance does not fully account for differences in $J_{INTERNAL}$. The additional processes influencing O_3 flux are collectively referred to as residual resistance and involve any physiological or biochemical processes that affect either the chemical potential gradient of the pollutant (derivative) or the pathway's diffusive resistance.

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